FORM PTO-1390 (REV. 9-2001) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY 'S DOCKET NUMBER	
P/2432-44	
U.S. APPLICATION NO. (If known, see 37 CFR 1.5	10/009709

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/SE00/00930	11 May 2000	17 May 1999

TITLE OF INVENTION: PREVENTION OF DENTAL CARIES

APPLICANT(S) FOR DO/EO/US: Nicklas STROMBERG et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [x] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. [x] The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. [x] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not communicated by the International Bureau).
   b. [x] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. [x] is attached hereto.
   b. [ ] has been previously submitted under 35 U.S.C. 154(d)(4).
7. [ ] Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [x] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). - unsigned
10. [ ] An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [x] A FIRST preliminary amendment.
14. [ ] A SECOND or SUBSEQUENT preliminary amendment.
15. [ ] A substitute specification.
16. [ ] A change of power of attorney and/or address letter.
17. [xx] A computer-readable form of the sequence listing in accorda
18. [ ] A second copy of the published international application und
19. [ ] A second copy of the English language translation of the inte
20. [x] Other items or information:
    PEFS Print form.
    3 sheets of drawings.
    Sequence Listing with disk.

EXPRESS MAIL CERTIFICATE

I hereby certify that this correspondence is being deposited with the United States Postal Service as Express Mail Post Office to Addresses (mail label EL334617104US in an envelope addressed to: Asst. Commissioner for Patents, Washington, D.C. 20231. on November 16, 2001

Dorothy Jenkins
Name of Person Mailing Correspondence

Dorothy Jenkins
Signature

November 16, 2001
Date of Signature

531 Rec'd PCT/PTO 19 NOV 2001

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
10/089709	PCT/SE00/00930	P/2432-44

21. [X] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):

		CALCULATIONS PTO USE ONLY
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1040.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	$890.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$740.00	
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$710.00	
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	$100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =		$ 1040.00
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		$ 1040.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	17 - 20 =	0	x $18.00	$
Independent claims	1 - 3 =	0	x $84.00	$
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $280.00	$
TOTAL OF ABOVE CALCULATIONS =				$ 1040.00
[ ] Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.			+	$
SUBTOTAL =				$ 1040.00
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$
TOTAL NATIONAL FEE =				$ 1040.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$
TOTAL FEES ENCLOSED =				$ 1040.00
			Amount to be refunded:	$
			charged:	$

a. [X] A check in the amount of $ 1040. to cover the above fees is enclosed. Check No. 7364

b. [ ] Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 15-0700. A duplicate copy of this sheet is enclosed.

d. [ ] Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

OSTROLENK,FABER,GERB & SOFFEN, LLP
1180 Avenue of the Americas
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Edward A Meilman
SIGNATURE

Edward A. Meilman
NAME

24,735
REGISTRATION NUMBER

P/2432-44

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

Nicklas Strömberg et al.

Serial No..

Filed:

For: PREVENTION OF DENTAL CARIES

Date: November 16, 2001

Group Art Unit:

Examiner:

Asst. Commissioner for Patents

Washington, D.C. 20231

AMENDMENT/SUBMISSION

Prior to examination, please amend the application as follows.

FEE CALCULATION

Any additional fee required has been calculated as follows:

_____ If checked, "Small Entity" status is claimed.

	NO. CLAIMS AFTER AMENDMENT		HIGHEST NO. PREVIOUSLY PAID FOR		EXTRA PRESENT		RATE	ADDIT. FEE
TOTAL	17	MINUS	20	* =	0	X	($9 SE or $18)	$
INDEP.	1	MINUS	3	** =	0	X	($42 SE or $84)	$
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM						X	($140 SE or $280)	$

* not less than 20** not less than 3 TOTAL $ -0-

If any additional payment is required, a check which includes the calculated fee of $_____ (OFGS Check No. _____) is attached.

In the event the actual fee is greater than the payment submitted or is inadvertently not enclosed or if any additional fee during the prosecution of this application is not paid, the Patent Office is authorized to charge the underpayment to Deposit Account No. 15-0700.

CONTINGENT EXTENSION REQUEST

If this communication is filed after the shortened statutory time period had elapsed and no separate Petition is enclosed, the Commissioner of Patents and Trademarks is petitioned, under 37 C.F.R. §1.136(a), to extend the time for filing a response to the outstanding Office Action by the number of months which will avoid abandonment under 37 C.F.R. §1 135. The fee under 37 C.F.R. § 1.17 should be charged to our Deposit Account No. 15-0700.

AMENDMENTS

_X_ If checked, amendment(s) to the specification and/or claims are submitted herewith.

Claims:

Please amend claims 5, 6 and 8 and add new claims 9-17 pursuant to 37 C.F.R. § 1.121(c)(i) as set forth in the "clean" version attached hereto as Appendix A. Entry is respectfully requested. A version with markings to show the changes made pursuant to 37 C.F.R. § 1.121(c)(ii) is attached hereto as Appendix B.

REMARKS/ARGUMENT

This Preliminary Amendment is being submitted to change the multiple dependent claims to single dependent claims in order to eliminate the improper multiple dependent claims and to reduce the government filing fee.

EXPRESS MAIL CERTIFICATE

I hereby certify that this correspondence is being deposited with the United States Postal Service as Express Mail to Addressee (mail label # EL534617104US) in an envelope addressed to: Asst. Commissioner for Patents, Washington, D.C. 20231, on November 16, 2001

Dorothy Jenkins
Name of Person Mailing Correspondence

Dorothy Jenkins
Signature

November 16, 2001
Date of Signature

Respectfully submitted,

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APPENDIX A

"CLEAN" VERSION OF EACH PARAGRAPH/SECTION/CLAIM
37 C.F.R. § 1.121(b)(ii) AND (c)(i)

CLAIMS (with indication of amended or new):

5. (Amended) A method of preventing dental caries comprising the oral administration of a prevention-effective amount of the peptide of claim 1.

6. (Amended) A composition for preventing dental caries comprising a prevention-effective amount of the oligopeptide of claim 1.

8. (Amended) The manufacture of a medicament for prevention of dental caries comprising a prevention-effective amount of the oligopeptide of claim 1.

9. (NEW) A method of preventing dental caries comprising the oral administration of a prevention-effective amount of the peptide of claim 2.

10 (NEW) A method of preventing dental caries comprising the oral administration of a prevention-effective amount of the peptide of claim 3.

11. (NEW) A method of preventing dental caries comprising the oral administration of a prevention-effective amount of the peptide of claim 4.

12. (NEW) A composition for preventing dental caries comprising a prevention-effective amount of the oligopeptide of claim 2.

13 (NEW) A composition for preventing dental caries comprising a prevention-effective amount of the oligopeptide of claim 3.

14. (NEW) A composition for preventing dental caries comprising a prevention-effective amount of the oligopeptide of claim 4.

15. (NEW) The manufacture of a medicament for prevention of dental caries comprising a prevention-effective amount of the oligopeptide of claim 2.

16. (NEW) The manufacture of a medicament for prevention of dental caries comprising a prevention-effective amount of the oligopeptide of claim 3.

17. (NEW) The manufacture of a medicament for prevention of dental caries comprising a prevention-effective amount of the oligopeptide of claim 4.

APPENDIX B

VERSION WITH MARKINGS TO SHOW CHANGES MADE

37 C.F.R. § 1.121(b)(iii) AND (c)(ii)

CLAIMS:

5. (Amended) A method of preventing dental caries comprising the oral administration of a prevention-effective amount of the peptide of [any of claims] claim 1[-4].

6. (Amended) A composition for preventing dental caries comprising a prevention-effective amount of the oligopeptide of [any of claims] claim 1[-4].

8. (Amended) The manufacture of a medicament for prevention of dental caries comprising a prevention-effective amount of the oligopeptide of [any of claims] claim 1[-4].

P/2432-44

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of
Nicklas Strömberg et al.
Serial No.: Not Yet Known
Filed: Herewith
For: PREVENTION OF DENTAL CARIES

Date: November 16, 2001
Group Art Unit: --
Examiner: --

Assistant Commissioner for Patents
Box Patent Application
Washington, D.C. 20231

STATEMENT IN ACCORDANCE WITH 37 C.F.R. §1.821(f)

Sir:

Pursuant to 37 C.F.R. §1.821(f), I hereby certify that the contents of the sequence listings enclosed in the above-identified patent application papers and the contents of the computer readable form of the sequence listings are the same.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any parent issued thereon.

Respectfully submitted,

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PREVENTION OF DENTAL CARIES

FIELD OF THE INVENTION

The present invention relates to a means for preventing dental caries, a corresponding method, and compositions incorporating the means.

BACKGROUND OF THE INVENTION

Proline-rich proteins (PRPs) comprise about 70% of the proteins in saliva. They can be divided into acidic, basic, and glycosylated PRPs encoded by six clustered genes on chromosome 12p13.2. PRPs are potential determinants of host susceptibility to dental caries. Large multifunctional 150-residue PRPs (Lamkin M S and Oppenheim F G, Crit Rev Oral Biol Rev 4 (1993) 251-9) are known to adsorb to hydroxyapatite surfaces and inhibit calcium phosphate precipitation, modulate hydroxyapatite crystal formation as well as mediate adhesion of commensal *Actinomyces* and *Streptococcus* species to tooth surfaces. In addition, they bind to and inactivate ingested plant polyphenols (tannins). The proline-poor N-terminal 30 residue domain confers hydroxyapatite- and calcium-binding, while the proline-rich middle/C-terminal domain binds bacteria via the ProGln-terminus and tannins via proline-rich repeats.

The polymorphism of acidic PRPs involves allelic and post-translational variants; several common allelic (large PRP-1, PRP-2, Db-s, PIF-s and Pa) and post-translational (small PRP-3, PRP-4, Db-f and PIF-f) variants have been described. In regard of biological properties the acidic PRP variants differ somewhat, and large and small acidic PRPs largely,

between each other. The small 106 residue acidic PRPs resulting from proteolytic cleavage at Arg106-Gly107 display poor bacterial adhesion activity but high affinity for hydroxyapatite surfaces. Both large and small acidic PRPs are secreted from the acinus cells. After secretion, the acidic PRPs are rapidly enriched on tooth surfaces and degraded as a consequence of bacterial proteolysis. Also, multiple phosphorylated peptides reminiscent of PRPs have been identified in saliva and found to possess increased affinity for hydroxyapatite surfaces. Some studies (Kousvelari E E et al., J Dent Res 59 (1980) 1430-8), though not others (Mandel I D & Bennick A, J Dent Res 62 (1980) 943-5), have associated this proteolytic activity to gingivitis and rate of plaque formation.

Arg catabolism to ammonia has been suggested to be a characteristic of streptococcal biotypes with tooth protective properties (Andersson C et al. Infect Immun 43 (1984) 555-60; Rogers AH, Aust Dent J 35 (1990) 468-71. Wijeyeweera R L& Kleinberg, Arch Oral Biol 34 (1989) 55-64; ibid. 43-53). Actually, Arg can control the ecological relationship between *S. sanguis* and *S. mutans* (van der Hoeven J S et al., J Dent Res 63 (1984) 389-92. Rogers A H et al., Oral Microbiol Immunol 2 (1987) 172-82), and caries-susceptible and caries-resistant subjects differ in ability to raise pH after an acidification (Abelson D C & Mandel I D, J Dent Res 60 (1981) 1634-8; Kleinberg I et al., Proc. "Microbial aspects of dental caries". Sp. Supp. Microbiol. Abstracts. IRL, Washington, D.C., 1976, 433-464 pp).

Arg catabolism is present in *S. sanguis*, *S. gordonii*, *S. anginosus* and *S. mitis*, while it is absent in *S. oralis* and cariogenic *S. mutans* and *S. sobrinus* (Kilian M et al., Int J

Syst Bacteriol 39 (1989) 471-484). Arg-containing peptides, notably sialin (Coulter W A et al., Biochem Soc Trans 18 (1990) 337-8) may act as a local pH-buffering agents by bacterial catabolism of Arg to ammonia via the arginine deaminase pathway (Floderus E et al., APMIS 98 (1990) 1045-52).

Degradation of acidic PRPs releases oligopeptides which are transported intra-cellularly and metabolized for nutritional reasons (Kunji E R et al., Antonie Van Leeuwenhoek 70 (1996) 187-221). Gram-negative bacteria colonizing the gingival pocket, such as *Porphyromonas gingivalis*, express a broad range of proteases in utilizing proteins as a major source of energy. In contrast, commensal streptococci and actinomycetes, which dominate on oral mucosal and tooth surfaces, express multiple glycosidases in utilizing carbohydrates as a major energy source. Nevertheless, protease and peptidase activities, such as a trypsin-like serine endoprotease in S. oralis, S-IgA1 proteases in *S. sanguis* and *S. oralis* and yet uncharacterized proteolytic activity, are present in *Streptococcus* and *Actinomyces* species. However, little is known about the degradation of acidic PRPs by these commensal and early colonizing bacteria.

While a vast amount of knowledge about saliva constitutents and their possible role in processes leading to dental caries has been gathered, that knowledge up to know resulted in only few practically applicable propositions of how to efficiently prevent dental caries.

OBJECTS OF THE INVENTION

It is an object of the invention to provide a means for protecting dental surfaces against caries.

It is another object of the invention to provide a corresponding method.

It is a further object of the invention to provide a composition for protecting dental surfaces against caries.

Still other objects of the invention will become apparent from the following description of the invention and preferred embodiments thereof, as well as from the appended claims.

DESCRIPTION OF THE INVENTION

The present invention is based on the insight that commensal *Actinomyces* and *Streptococcus* species transform acidic PRPs to small-size peptides, such as pentapeptides. These small-size peptides are transformed into ammonia by the action of certain oral bacteria. The ammonia thus formed protects raises the pH at the dental surface and therby protects the surface against caries.

In this transformation Arg-containing oligopeptides are released and catabolized to ammonia at or near the dental surface. Ammonia increases the pH at the dental surface and thereby counteracts caries which is promoted by a low pH in the oral cavity.

The present invention is also based on the insight that the aforementioned transformation gives rise to peptides with terminal proline or proline-glutamine entities having

affinity to dental surfaces and thus competitively protecting them.

Degradation of acidic PRPs by *Streptococcus* and *Actinomyces* species comprises the following features:
(i) step-by-step the large 150 residue PRP-1 protein is degraded into intermediate size peptides and finally into smaller oligopeptides and amino acids. Cell-bound endoproteases with broad substrate specificities are involved in the initial stages of this degradation;
(ii) cleavage of acidic PRPs occurs preferentially in the middle/C-terminal proline-rich 96-140 region; the 106 residue PRP-3 is relatively resistant to cleavage, PRP-3-like peptides being generated by all strains and a series of fragments in region 96-140. Both endoprotease specificity *per se* and intrinsic properties of acidic PRPs, such as the extended structure of proline-rich stretches, explains this regional preference of cleavage;
(iii) degradation of PRP-1 releases oligopeptides both immediately and after prolonged degradation. The immediate release of an Arg106Gly107Arg108Pro109Gln110 pentapeptide is indicated by the N-terminal 105 residue Pyr1-Pro104Pro105 and the C-terminal 40 residue Gly111-Pro149Gln150 peptides initially generated by *S. gordonii* strain SK12.

Mass spectroscopy identified a N-terminal pyroglutamatic acid (Pyr) post-translational modification of acidic PRPs that blocks sequencing by Edman degradation. The peptide structures derived from degradation of the 150 residue PRP-1 by *S. gordonii* strain SK12 indicate:
(i) the instant release of an Arg106Gly107Arg108Pro109Gln110 pentapeptide together with a N-terminal 105 residue Pyr1-

Pro104Pro105 and a C-terminal 40 residue Gly111-Pro149Gln150 peptide;

(ii) the generation of multiple middle/C-terminal peptides with ProGln-termini;

(iii) the cleavage at peptide bonds formed at Pro or Gln residues: Pro95-Pro96, Pro104-Pro105, Pro105-Arg106, Gln110-Gly111, Gln131-Gly132, Pro135-Gln136, Gln136-Gly137, and Gln141-Gly142.

Degradation of acidic PRPs by *S. gordonii* SK12 initially generates a pentapeptide, Arg106Gly107Arg108Pro109Gln110, containing two of the five Arg residues in PRP-1.

The N-terminal Glu1-Pro104Pro105 peptide derived from PRP-1 by S. gordonii SK12 lacks the bacterial adhesion-promoting ProGln-terminus, but contains the hydroxyapatite binding domain. Since small phosphorylated proline-rich peptides and PRP-3 display increased affinity for hydroxyapatite surfaces (Moreno E C et al., J Biol Chem 257 (1982) 2981-9; Madapallimattam G & Bennick A, Biochem J 270 (1990) 297-304; Lamkin M S et al., J Dent Res 75 (1996) 803-8), such N-terminal peptides can affect bacterial adhesion by occupying hydroxyapatite sites or altering the pellicle mosaic of binding structures. Proteolytic cleavage is known to unmask hidden binding structures (Gibbons R J et al., Arch Oral Biol 35 Suppl (1990) 107s-114s), and strains of *Actinomyces* species recognize diverse sites in acidic PRPs and statherin (Hallberg K et al., Oral Microbiol Immunol 13 (1998) 327-336; Li T et al., Infect Immun 67 (1999) 2053-9). Furthermore, acidic PRP-degradation generated multiple middle/C-terminal peptides with adhesion-active ProGln-termini, potentially inhibiting the adhesion of oral pathogens binding to acidic PRPs and statherin, i. e. *S.*

mutans (Gibbons R J & Hay D I, J Dent Res 68 (1989) 1303-7), *P. gingivalis* (Amano A et al., Infect Immun 62 (1994) 3372-80) and *Fusobacterium nucleatum* (Xie H et al., Oral Microbiol Immunol 6 (1991) 257-63). Commensal and pathogenic species display high and low avidity binding to acidic PRPs, respectively, and may respond differently to ProGln-terminating peptides. Alternatively, both the N-terminal and middle/C-terminal peptides could act as inhibitors or retarders of plaque formation in a broader sense.

While the inventors believe these explanations to be true they are of a hypothetical nature and should not be understood so as to limit the scope of the invention which is only determined by the appended claims.

According to the invention is disclosed an oligopeptide protecting against dental caries comprising two arginine residues selected from the group consisting of pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide and decapeptide. Particularly preferred is the pentapeptide ArgGlyArgProGln.

Also preferred is a penta- to decapeptide comprised by the sequence of amino acid 99 to amino acid 115 of the 150 residue PRP-1 protein:
GlyGlyHisProArgProProArgGlyArgProGlnGlyProProGlnGln,
with the provisio that it contains two or more Arg.

Also preferred are the following peptides:
ArgGlyArgProGln (residues 106-110);
ArgGlyArgProGlnGly (residues 106-111);
ArgGlyArgProGlnGlyPro (residues 106-112);
ArgGlyArgProGlnGlyProPro (residues 106-113);

ArgGlyArgProGlnGlyProProGln (residues 106-114);
ArgGlyArgProGlnGlyProProGlnGln (residues 106-115);
GlyGlyHisProArgProProArgGlyArg (residues 99-108);
GlyHisProArgProProArgGlyArg (residues 100-108);
HisProArgProProArgGlyArg (residues 101-108);
ProArgProProArgGlyArg (residues 102-108);
ArgProProArgGlyArg(residues 103-108);
ProProArgGlyArg (residues 104-108).

The peptides of the invention can be easily synthesized by solid phase or liquid phase methods known in the art.

According to the invention is disclosed a method of preventing dental caries comprising the oral administration of a prevention-effective amount of an oligopeptide comprising two arginine residues selected from the group consisting of pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide and decapeptide. Particularly preferred is the pentapeptide ArgGlyArgProGln for use in the method of preventing dental caries.

Also preferred for use in the method of preventing dental caries is a penta- to decapeptide comprised by the sequence of amino acid 99 to amino acid 115 of the 150 residue PRP-1 protein:GlyGlyHisProArgProProArgGlyArgProGlnGlyProProGlnGln, with the provisio that it contains two or more Arg.

Also preferred for use of preventing dental caries are the following peptides:
ArgGlyArgProGln (residues 106-110);
ArgGlyArgProGlnGly (residues 106-111);
ArgGlyArgProGlnGlyPro (residues 106-112);
ArgGlyArgProGlnGlyProPro (residues 106-113);

ArgGlyArgProGlnGlyProProGln (residues 106-114);
ArgGlyArgProGlnGlyProProGlnGln (residues 106-115);
GlyGlyHisProArgProProArgGlyArg (residues 99-108);
GlyHisProArgProProArgGlyArg (residues 100-108);
HisProArgProProArgGlyArg (residues 101-108);
ProArgProProArgGlyArg (residues 102-108);
ArgProProArgGlyArg(residues 103-108);
ProProArgGlyArg (residues 104-108).

According to the invention is disclosed a composition for preventing dental caries comprising a prevention-effective amount of an oligopeptide comprising two arginine residues selected from the group consisting of pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide and decapeptide, and a suitable carrier. Particularly preferred is the pentapeptide ArgGlyArgProGln. Suitable carriers include state-of-the-art toothpaste and mouthwash compositions but also chewing-gums, lozenges, and the like.

Also preferred for use in the composition for preventing dental caries is a penta- to decapeptide comprised by the sequence of amino acid 99 to amino acid 115 of the 150 residue PRP-1 protein:
GlyGlyHisProArgProProArgGlyArgProGlnGlyProProGlnGln,
with the provisio that it contains two or more Arg.

Also preferred for use in the composition for preventing dental caries are the following peptides:
ArgGlyArgProGln (residues 106-110);
ArgGlyArgProGlnGly (residues 106-111);
ArgGlyArgProGlnGlyPro (residues 106-112);
ArgGlyArgProGlnGlyProPro (residues 106-113);
ArgGlyArgProGlnGlyProProGln (residues 106-114);
ArgGlyArgProGlnGlyProProGlnGln (residues 106-115);

GlyGlyHisProArgProProArgGlyArg (residues 99-108);
GlyHisProArgProProArgGlyArg (residues 100-108);
HisProArgProProArgGlyArg (residues 101-108);
ProArgProProArgGlyArg (residues 102-108);
ArgProProArgGlyArg(residues 103-108);
ProProArgGlyArg (residues 104-108).

Since the caries-protecting peptides according to the invention are peptides produced by the human body or correspond to sequences thereof they are well tolerated. Optimal caries-protecting amounts of these peptides can be easily determined by experiment because the absence of important toxicity restraints. Single doses from 0.1 mg to 1 g will be appropriate for most applications.

The invention will be better understood by reference to a drawing and the following description of preferred embodiments thereof.

DESCRIPTION OF THE DRAWING

Fig. 1 illustrates the native alkaline PAGE patterns of N-terminal acidic peptides from degradation of acidic PRPs (PRP-1 and PRP-3) by strains of commensal Streptococcus and *Actinomyces* species;

Fig. 2 illustrates the gel filtration patterns of fragments formed by degradation of PRP-1 by strains representative for each of the four degradation patterns identified among streptococci and actinomycetes;

Fig. 3a illustrates the gel filtration patterns of peptide fragments from the degradation of PRP-1 by *S. gordonii* strain SK12 for different time periods;

Fig. 3b illustrates the identification by mass spectrometry of fragments separated using either gel filtration (peaks 1-4, cf. Fig. 3a) or peptide gel filtration (peak 5).

DESCRIPTION OF PREFERRED EMBODIMENTS

EXAMPLE 1. **Isolation of acidic PRPs.** Parotid saliva was collected from three subjects homozygous for PRP-1 and PIF-s using Lashley cups and mild acidic lozenge stimulation. After pooling of the salivas and dilution (1:1 by volume) with 50 mmol/L Tris-HCl, 25 mmol/L NaCl, pH 8.0 (Tris-HCl buffer), the saliva sample was subjected to DEAE-Sephacel column chromatography (15 x 1.6 cm, Pharmacia, Uppsala, Sweden) using a linear gradient of 25 to 1000 mmol/L NaCl in Tris-HCl buffer. The protein fraction containing the acidic PRPs (PRP-1/PIF-s and PRP-3/PIF-f) was concentrated via ultrafiltration using a Centriprep 10 concentrator (Amicon Inc., Beverly, MA), and subjected to gel filtration (HiLoad™ 26/60 Superdex S-200 Prep grade column, Pharmacia) in 20 mmol/L Tris-HCl, 500 mmol/L NaCl, pH 8.0. The resolved protein fractions (PRP-1/PIF-s and PRP-3/PIF-f) were dialyzed against Tris-HCl buffer (Spectra/Pore membrane No. 4, Spectrum Medical Industries, Inc., Houston, TX). Each protein fraction (PRP-1/PIF-s and PRP-3/PIF-f) was finally purified on a Macroprep high Q column (15 x 1.6 cm, Bio-Rad, Hercules, CA) using a linear gradient of 25 to 1000 mmol/L NaCl in 50 mmol/L Tris-HCl, pH 8.0. The purified proteins were extensively dialyzed against water, lyophilized and stored at -20ºC.

EXAMPLE 2. **Bacterial strains and culturing**. The origins and species designations of strains of *Actinomyces naeslundii*, *Actinomyces odontolyticus*, *A. naeslundii genospecies* 1 strain ATCC 12104, genospecies 2 strain T14V, *A. odontolyticus* strain PK984 and *A. viscosus* strain 19246 are as previously described (Hallberg K et al, see above; Hallberg K et al., Oral Microbiol Immunol 13 (1989) 188-192; Li et al., 1999, see above). The strains of *S. anginosus*, *S. gordonii*, *S. mitis*, *S. oralis* and *S. sanguis* (provided by Dr. M. Kilian, Department of Oral Biology, Royal Dental College, Aarhus, Denmark) have been described (Kilian et al., 1989; Hsu et al., 1994). *S. mutans* strains Ingbritt, JBP and NCTC 10449, *S. sobrinus* strain SL-1 and *Porphyromonas gingivalis* strains W83 and ATCC 33277 were provided by Dr. J. Carlsson, Department of Oral Biology, Umeå Universìty, Sweden. All strains were grown overnight on Columbia-II-agar base plates (Becton Dickinson and Company, Cockeysville, MD), supplemented with 30 ml of a human erythrocyte suspension per liter, at 37ºC in an atmosphere with 5 % CO2. Growth of *S. gordonii* strain SK12 identified two morphological types, one forming flat (strain SK12-I) and the other convex (strain SK12-II) colonies. Strain SK12-I (the flat colony-forming type) was selected for the degradation experiments. Neither whole cell soluble protein patterns, as analyzed by SDS-PAGE, nor acidic PRP cleavage patterns distinguished between the two types. The strains intended for degradation experiments were then grown at 37ºC for 18 h in 5 mL trypticase soy bean glucose limiting broth (1.7% peptone, 0.3% soy peptone, 0.15% yeast extract, 12.5 mmol/L glucose, 12.5 mmol/L NH_4HCO_3 in 1 mol/L NaH_2PO_4/K_2HPO_4 buffer, pH 7.3) in an atmosphere with 5% CO_2. The cells were pelleted by centrifugation at 17,000 x g for 5 min, washed

twice in 0.43% NaCl, 0.042% KCl, 0.1% Na_2HPO_4, 0.1% KH_2PO_4, 1% glycerophosphate disodium salt, 0.024% $CaCl_2$, and 0.01% $MgCl_2.H_2O$ (M-DIL buffer), re-suspended in M-DIL buffer at a concentration of 2 x 109 cells/mL. The bacterial cells (and cell-free supernatant after pelleting of bacteria by centrifugation) were kept on ice prior to degradation experiments. The strains intended for adhesion tests were metabolically labeled by adding 35S-methionine (200 *Ci, Tran 35S-Label, ICN Pharmaceuticals Inc., Irvine, CA) to bacteria suspended in 100 μL 10 mmol/L Na_2HPO_4, NaH_2PO_4, 0.137 M NaCl, pH 7.2, (PBS) prior to growth on Columbia-II-agar plates.

EXAMPLE 3. **Degradation of acidic PRPs.** Equal volumes (200 μL) of purified protein (0.6 mg/mL) and bacteria (2 x 109 cells/mL), both dissolved in M-DIL buffer, were mixed and incubated at 37ºC for 15 min, 4 h, 20 h and 1 week. After pelleting of the bacteria by centrifugation at 17,000 x g for 10 min, the supernatants were aliquoted, lyophilized and stored at -80ºC prior to analysis. In some experiments, cell free enzyme supernatants and parotid saliva from defined acidic PRP phenotypes were used following the same protocol. The saliva was collected as described above and sterilized by filtering (0.20 μm Minisart filter, Sartorius, Göttingen, Germany).

EXAMPLE 4. **Native alkaline PAGE.** Native alkaline polyacrylamide gel electrophoresis was performed essentially as described (Azen and Yu, 1984). The lyophilized supernatants were dissolved in 50 μL sample buffer containing 1% glycine, 10% glycerol and 0.025% bromophenol blue and centrifuged at 17,000 x g for 10 min. The electrophoresis was performed using precast Tris-glycine

7.5% resolving gels with 4% stacking gels (Bio-Rad) at 100 V for 20 min followed by 175 V for 1 h 30 min in a buffer containing 0.038 M Tris-glycine, pH 8.4. The gels were stained with 0.1% Coomassie Blue R (Serva Feinbiochemica, Heidelberg, Germany) in 20% trichloroacetic acid (Merck, Darmstadt, Germany) for 16 h, destained in 2% acetic acid for 16 h and finally soaked in water for 2 h.

EXAMPLE 5. **Densitometry.** The degree of acidic PRP degradation was quantified by densitometry of native alkaline PAGE gels using a Model GS-700 Imaging densitometer and the Molecular Analyst Software (Bio-Rad). The degree of degradation of PRP-1 and PRP-3 was scored from 0 to 5 according to the following criteria: 0 = 0<10% , 1 = 10<20%, 2 = 20<40%, 3 = 40<60%, 4 = 60<80%, 5 = 80<100% reduction of PRP-1 or PRP-3 at 4 h of incubation. In the case of certain strains, score 1 denotes the formation after 20 h of incubation of peptide fragments similar to those generated after 4 h by the strains with a high PRP degradation rate (Table 1).

EXAMPLE 6. **Gel filtration.** The lyophilized supernatants obtained by incubation of bacteria with acidic PRPs were dissolved in 200 μL 20 mM Tris-HCl, pH 8.0, containing 0.5 M NaCl and subjected to gel filtration on Superose 12 HR 10/30 or a Superdex Peptide HR10/30 (Pharmacia) columns, equilibrated in 20 mM Tris-HCl, pH 8.0. The flow rate was 0.2 mL/min and the absorbance was monitored at 214 nm. Fractions of 1 mL were collected.

EXAMPLE 7. **Hydroxyapatite assay.** Adherence of 35S-methionine labeled bacteria to acidic PRP-1 was measured by the

hydroxyapatite assay as previously described (Carlén et al., 1998).

EXAMPLE 8. **Mass spectrometry.** All mass spectrometric data were acquired on a hybrid quadrupole time-of-flight (Q-ToF) mass spectrometer (Micromass, Manchester, UK). A Z-configured nano spray source was used and samples were introduced via gold-coated spraying needles (Protana, Odense, Denmark). Detection was all times in the positive ion mode. Needles were opened manually under a light-microscope resulting in an opening of approximately 5 μm. Before analysis, remaining contaminants and salt were removed by applying the samples to nano-columns; Gel-loader tips (Eppendorf, Hamburg, Germany) packed with POROS R3 reverse phase resin (PerSeptive Biosystems, Framingham, MA, USA) to a bed volume of approximately 200 nL. Normally, 5 μL of sample was applied to the resin followed by washing with approximately 30 μL 0.1% TFA and elution with 60% CH3CN in 1% acetic acid directly into the nano-spray needle.

EXAMPLE 9. **N-terminal sequence analysis.** Edman degradation was performed directly on proteins blotted (Transblot, Bio-Rad) onto PVDF-membranes using an Applied Biosystems 477A pulsed liquid phase sequencer (Foster City, CA) with an on-line PTH 120A analyzer. Sequence analysis was performed with cycle programs adapted to the reaction cartridges and chemicals from the manufacturer. Yields were calculated from sequenced standard *-lactoglobulin.

EXAMPLE 10. **Substrate specificity.** The substrate specificity of bacterial strains was measured as previously described (Erlanger et al., 1961). Briefly, 25 μL of bacterial suspension (5 x 109 cells/mL in M-DIL buffer) was diluted

with 25 μL of 0.1 mol/L Tris-HCl, pH 7.0, followed by addition of 50 μL of B-Arg-pNA (Sigma, St. Louis, MO) and Z-Gly-Pro-pNA (Bachem, Bulendorf, Switzerland) (8.0 mmol/L in DMSO). After incubation at 37ºC for 16 h, the extent of cleavage was measured by the absorbance at 414 nm.

EXAMPLE 11. **Degradation of acidic PRPs by commensal Streptococcus and Actinomyces species.** Strains of *Streptococcus* and *Actinomyces* species were tested for degradation of acidic PRPs (PRP-1 and PRP-3) by co-incubation of bacteria and protein followed by native alkaline PAGE (Table 1, Fig. 1). Degradation of PRP-1 occurred with *S. oralis* (1 of 4 strains), *S. mitis* (1 of 4 strains), *S. gordonii* (3 of 4 strains), *S. sanguis* (4 of 4 strains), *S. anginosus* (3 of 4 strains) and *A. odontolyticus* (4 of 7 strains), but not with *S. mutans* (3 strains), *S. sobrinus* (1 strain), *A. naeslundii* genospecies 1 (6 strains) and genospecies 2 (7 strains) or *A. viscosus* (1 strain). *S. gordonii* and *A. odontolyticus* displayed high PRP-1 cleavage rates (scores 4 and 5), while other species had moderate (scores 2 and 3) to low (score 1) cleavage rates. While most species showed high PRP-1 relative to PRP-3 cleavage rates, *A. odontolyticus* displayed equal PRP-1 and PRP-3 cleavage rates. Degradation of PRP-1 also occurred in cell-free supernatants (Table 1), and *S. gordonii* strain SK12 (with a high PRP-1 cleavage rate) degraded PRP-1, PRP-2, Db-s, PIF-s and Pa in parotid saliva from defined donors (data not shown).

Both streptococci adhering to and those not adhering to PRP-1 degraded acidic PRPs (Table 1). While *A. naeslundii* genospecies 1 and 2 bound to but did not degrade acidic PRPs, the opposite was true for *A. odontolyticus*. In

addition, both S-IgA1 protease positive and negative strains degraded acidic PRPs (Table 1). Thus, no obvious relationship was found between PRP degradation and adhesion to acidic PRPs or S-IgA1 protease activity.

EXAMPLE 12. **Time-dependency of acidic PRP degradation**. The native alkaline PAGE patterns from degradation of acidic PRPs for different times indicated a time-dependency of degradation for all strains (Fig. 1). The time-dependency of degradation of PRP-1 by *S. gordonii* strain SK12 was further analyzed by gel filtration of fragments formed after different times of incubation (Fig. 3a). Degradation of PRP-1 (peak 1) for 15 min generated a peptide eluting similar to PRP-3 (peak 2) and later-eluting, smaller peptides (peak 3). Prolonged degradation (20 h) completely converted PRP-1 into the peptide eluting similar to PRP-3 (peak 2) and even later-eluting peptides (peak 4). Even further degradation (1 week) completely transformed all peptides (except for the one eluting similar to PRP-3) into oligopeptides and amino acids (peak 5), as identified using peptide gel filtration (data not shown).

EXAMPLE 13. **Identity and structural features of acidic PRP-derived peptides.** The peptides generated from PRP-1 by *S. gordonii* strain SK12 were identified by mass spectrometry of the peptide peaks obtained by gel filtration (Figs 2 and 3). An N-terminal 105 residue peptide Pyr1-Pro104Pro105 (peak 2) and a C-terminal 40 residue peptide Gly111-Pro149Gln150 (peak 3) were identified after initial (15 min) cleavage of PRP-1 (Fig. 3b). A series of 15-47 residue peptides (peak 4; Pro96-Pro109Gln110, Gly111-Pro130Gln131, Gly111-Pro134Pro135, Gly111-Pro135Gln136, Gly111-Pro140Gln141, Gly111-Pro149Gln150 and Pro104-Pro149Gln150) were identified

after prolonged degradation (20 h). In addition, a pyroglutamatic acid, Pyr, was found at the N-terminal residues of PRP-1 and PRP-3 (Fig. 3b), explaining our difficulties in sequencing of N-terminal fragments by Edman degradation.

EXAMPLE 14. **Patterns of acidic PRP degradation.** The peptide profiles displayed by native alkaline PAGE distinguished four major degradation patterns, I-IV (Table 1, Fig. 1). While all four patterns contained N-terminal peptides migrating close to PRP-3, the peptide banding patterns were unique to a particular strain or species (Fig. 1, Table 1). Both species specific (*A. odontolyticus* and *S. gordonii*) and multiple patterns within a species (*S. anginosus* and *S. sanguis*), as well as a sharing of patterns between species, were observed. In addition, gel filtration of the degradation products obtained by a strain representative of each pattern verified somewhat deviating profiles, containing large (70-75 min retention) and intermediate (80-95 min retention) peptides (Fig 1b). To investigate the substrate specificities associated with acidic PRP degradation, strains representative of each degradation pattern were tested for truncation of Pro- and Arg-containing chromogenic substrates. The strains did not cleave Z-Gly-Pro-pNA, which is a substrate for prolyl endoproteases (Blumberg et al., 1980), or B-Arg-pNA, while *P. gingivalis* strain ATCC 33277 cleaved both substrates.

EXAMPLE 15. **Lozenge**. A solution of ArgGlyArgProGln 'acetate' was prepared by dissolving ArgGlyArgProGln in water and adding acetic acid to pH 6.5. The aqueous solution was freeze-dried and the powder thereby obtained mixed with 150 g of polyyethylene glycol 8000, 150 g of

microcrystalline cellulose, 600 g of mannitol, 10 g of stearic acid are milled to pass a 40 mesh sieve. The mixture is fed to a tablet press to produce 1 g tablets.

EXAMPLE 16. **Chewable tablet**. 900 g mannitol and 5 g sodium saccharin are screened through a 40-mesh screen and blended thoroughly with 40 g ArgGlyArgProGln acetate prepared as described above. A binder solution of 20 g of acacia and 50 g of gelatin in 500 ml water was prepared separately. The powder was wet granulated using 200 ml of binder solution for 1000 powder. After drying overnight at 75°C the granules were screened through a 12 mesh screen, mixed with 1 g of peppermint oil adsorbed on 3 g of colloidal silica and 25 g magnesium stearate. From this mixture 1 g tablets were compressed to a hardness of 12 kg.

EXAMPLE 17. **Toothpaste**. A suitable toothpaste base for incorporating the compounds of the invention is described in U.S. Patent No. 3,935,305 (Delaney et al.) which is incorporated herein by reference; in particular, see Example 1. A suitable amount of ArgGlyArgProGln acetate (for preparation, see above) is 1% by weight of base.

EXAMPLE 18. **Mouthwash**. A suitable mouthwash base for incorporating the compounds of the invention is described in U.S. Patent No. 5,145,664 (Thompson) which is incorporated herein by reference; see Example 1. A suitable amount of ArgGlyArgProGln acetate (for preparation, see above) is 1% by weight of base.

LEGENDS TO FIGURES

Fig 1. Native alkaline PAGE patterns of N-terminal acidic peptides from degradation of acidic PRPs (PRP-1 and PRP-3) by strains of commensal Streptococcus and *Actinomyces* species. Shown are the degradation patterns of strains representative for each of the four degradation patterns (I-IV) identified among isolates of streptococci and actinomycetes (cf. Table 1). All strains (Table 1) were co-incubated with purified PRP-1 and PRP-3 for different times (15 min, 4h and 20 h) and analyzed for such peptide patterns.

Fig. 2. Gel filtration patterns of fragments formed by degradation of PRP-1 by strains representative for each of the four degradation patterns identified among streptococci and actinomycetes (cf. Table 1). The strains were incubated with PRP-1 for 15 min and 20 h. The retention times of purified PRP-1 and PRP-3 are indicated by vertical arrows.

Fig 3a. Gel filtration patterns of peptide fragments from degradation of PRP-1 by *S. gordonii* strain SK12 for different times. The numbering of the peaks refer to mass spectrometric identification of the corresponding peptide structures (cf. Fig. 3b). The vertical arrows denote the retention times of purified PRP-1 and PRP-3.

Fig. 3b. Identification by mass spectrometry of fragments separated using either gel filtration (peaks 1-4, cf. Fig. 3a) or peptide gel filtration (peak 5). The numbering of peptides by letters A-G denotes the corresponding signals in the mass spectrum. Pyr indicates a pyroglutamic acid. The mass numbers with an asterisk indicate average masses from de-convoluted mass spectra, while unlabeled mass numbers are consistent with the monoisotopic mass of the peptide.

Table 1. Degradation of acidic PRPs by *Streptococcus* and *Actinomyces* species

Species[a]	Strain[a]	Acidic PRP degradation[b]			PRP-1 adhesion[d]	IgA1 protease[e]
		PRP-1	PRP-3	Type[c]		
S. gordonii	SK 12, SK 184	5	1	I	-	-
	SK 120	5	0	I	+	-
	SK 33	0	0	-	-	-
S. sanguis	SK 85	4	0	I	-	+
	SK 112	2	1	II	+	+
	SK 37	2	1	II	-	+
	SK 162	2	1	III	-	+
S. mitis	SK 304	1	0	II	-	-
	SK 305	0	0	-	+	-
	SK 96, SK 142	0	0	-	-	-
S. anginosus	SK 215	3	1	III	-	-
	SK 52	1	1	II	-	-
	SK 63	1	0	II	-	-
	SK 218	0	0	-	-	-

	S. oralis	SK 143	1	0	IV	-	+
		SK 2, SK 92	0	0	-	-	+
		C 104	0	0	-	nt	+
	S. mutans	Ingbritt, JBP	0	0	-	-	nt
5		NCTC 10449	0	0	-	nt	nt
	S. sobrinus	SL-1	0	0	-	nt	nt
	A. odontolyticus	T-5-G	5	5	IV	+	nt
		T-1-K	2	1	IV	-	nt
		T-23-N,T-3-G	1[b]	1	IV	-	nt
10		T-21-N, T-22-N, PK 984	0	0	-	-	nt
	A. naeslundii genospecies 1						
		ATCC 12104, P-3-N, P-5-N, P-11-N, B-2-G, PK 947	0	0	-	-	nt
	A. naeslundii genospecies 2						
15		T14V, P-1-N, P-7-N, P-1-K, P-1-G, B-7-N, P-2-N	0	0	-	+	nt
	A. viscosus	19246	0	0	-	-	nt
	P. gingivalis	W83	5	5	-	-	+

20 a) The sources of the strains are given above.

b) Acidic PRP degradation was carried out by co-incubation of PRP-1 or PRP-3 and bacteria for 15 min, 4 h and 20 h followed by native alkaline PAGE (Fig.1). The degree of cleavage at 4 h was scored from 0 to 5 by densitometry: 0 = 0<10 %,
25 1 = 10<20 %, 2 = 20<40 %, 3 = 40<60 %, 4 = 60<80 %, and 5 = 80<100 % loss of acidic PRPs. Score 1 labeled with a superscript denotes the formation of degradation products. Cells free supernatant resulted in similar degradation patterns for most strains. 95 % confidence intervals of
30 densitometric runs corresponded to 8 % of mean.

c) The strains were classified into degradation patterns I-IV based on native alkaline PAGE peptide profiles (Fig.1). The type-I pattern displayed a peptide migrating just below PRP-3; The type-II pattern a peptide migrating just below PRP-3
35 and another between PRP-1 and PRP-3; The type-III pattern two peptides migrating just below and above PRP-3, one between PRP-1 and PRP-3 and a fourth just below PRP-1; and the type-IV pattern a peptide migrating identical to PRP-3 and another between PRP-1 and PRP-3 although closer to PRP-1 compared to
40 patterns II and III.

d) Adhesion of ^{35}S-labeled bacteria to PRP-1-coated hydroxyapatite beads. Adhesion exceeding 15 % of added cells was considered positive. Adhesion of *Actinomyces* was tested at 5µg/ml of PRP-1 and for other bacteria at 30 µg/ml of PRP-1. The criteria for positive binding and assay conditions differ from those in other studies of adhesion of streptococci and *P. gingivalis* (Hsu *et al.*, 1994).

e) S-IgA protease activity of commensal streptococci (Kilian *et al.*, 1989), *P. gingivalis* (Kilian, 1981) and *Actinomyces* species, which lack S-IgA1 protease activity (M. Kilian, personal communication).

Table 2. Simplified map of generated peptides in comparison with PRP-1.

Peak	Peptide		Mass (Da)
1	Pyr1----Pro149-Gln150		15 514.0*
2	Pyr1----Pro104-Pro105		11 005.5*
3	Gly111---Pro149-Gln150		3 930.0
4	Pro96----Pro109-Gln110	(A)	1 663.7
4	Gly111---Pro130-Gln131	(B)	2 082.1
4	Gly111---Pro134-Pro135	(C)	2 430.4
4	Gly111---Pro135-Gln136	(D)	2 558.4
4	Gly111---Pro140-Gln141	(E)	3 053.6
4	Gly111---Pro149-Gln150	(F)	3 930.0

4	Pro104---Pro149-Gln150 (G)	4	718.3
5	Oligopeptides/amino acids		

The potential cleavage sites at peptide bonds formed at Pro or Gln residues are given. The presumed Arg-containing pentapeptide is blown up, ProGln-termini are marked by black circles and the post-translational cyclization of the N-terminal Glu residue to a pyroglutamic acid residue is marked Pyr. The verification by mass spectrometry of phosphorylation of Ser at positions 8 and 22 is marked.

C l a i m s

1. An oligopeptide protecting against dental caries comprising two arginine residues selected from the group consisting of pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide and decapeptide.

2. The oligopeptide of claim 1 comprised by the sequence GlyGlyHisProArgProProArgGlyArgProGlnGlyProProGlnGln.

3. The oligopeptide of claim 2 ArgGlyArgProGln.

4. The oligopeptide of claim 2 selected from:
ArgGlyArgProGln;
ArgGlyArgProGlnGly;
ArgGlyArgProGlnGlyPro;
ArgGlyArgProGlnGlyProPro;
ArgGlyArgProGlnGlyProProGln;
ArgGlyArgProGlnGlyProProGlnGln;
GlyGlyHisProArgProProArgGlyArg;
GlyHisProArgProProArgGlyArg;
HisProArgProProArgGlyArg;
ProArgProProArgGlyArg;
ArgProProArgGlyArg;
ProProArgGlyArg.

5. A method of preventing dental caries comprising the oral administration of a prevention-effective amount of the peptide of any of claims 1-4.

6. A composition for preventing dental caries comprising a prevention-effective amount of the oligopeptide of any of claims 1-4 and a suitable carrier.

7. The composition of claim 6 wherein the carrier is selected from the group consisting of toothpaste, mouthwash chewing-gum, lozenge, chewable tablet.

8. The manufacture of a medicament for prevention of dental caries comprising a prevention-effective amount of the oligopeptide of any of claims 1-4.

Abstract

A penta- to decapeptide containing two or more Arg protecting against dental caries is comprised by the amino acid sequence GlyGlyHisProArgProProArgGlyArgProGlnGlyProProGlnGln. Also disclosed is a composition for preventing dental caries comprising an effective amount of the peptide, and a corresponding method of use.

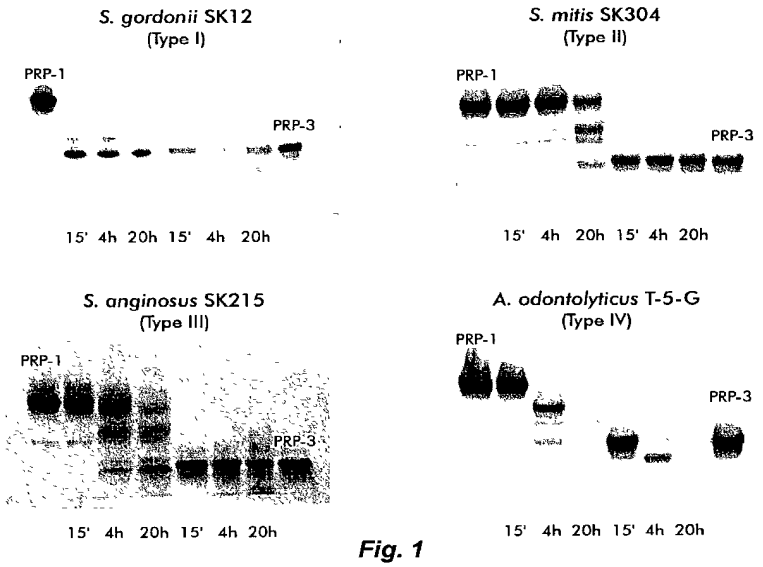
S. gordonii SK12
(Type I)
PRP-1
PRP-3
15' 4h 20h 15' 4h 20h
S. mitis SK304
(Type II)
PRP-1
PRP-3
15' 4h 20h 15' 4h 20h
S. anginosus SK215
(Type III)
PRP-1
PRP-3
15' 4h 20h 15' 4h 20h
A. odontolyticus T-5-G
(Type IV)
PRP-1
PRP-3
15' 4h 20h 15' 4h 20h

Fig. 1

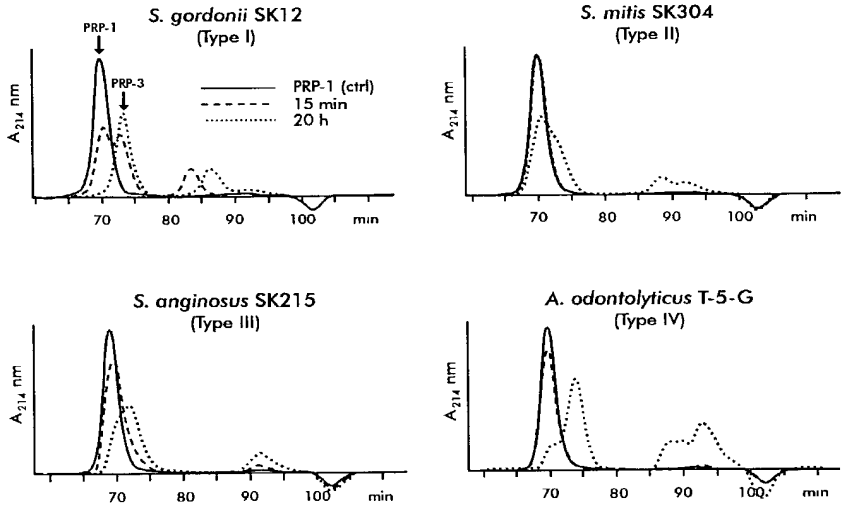
S. gordonii SK12
(Type I)
PRP-1
PRP-3
PRP-1 (ctrl)
15 min
20 h
A214 nm
70
80
90
100
min
S. mitis SK304
(Type II)
S. anginosus SK215
(Type III)
A. odontolyticus T-5-G
(Type IV)

Fig. 2

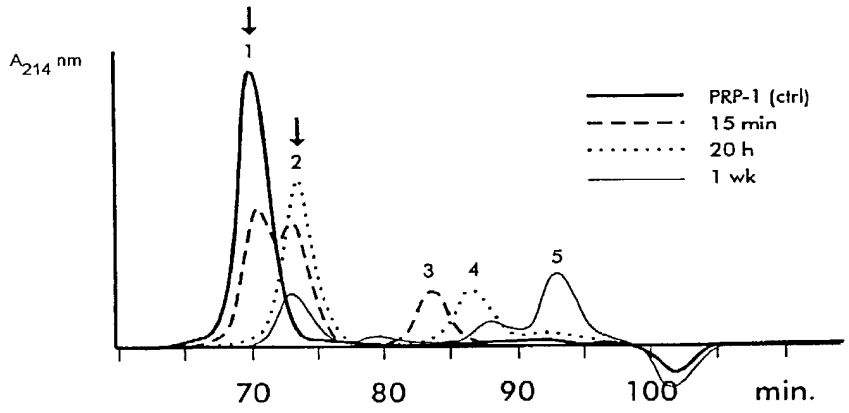
A214 nm
1
2
3
4
5
PRP-1 (ctrl)
15 min
20 h
1 wk
70
80
90
100
min.

Fig. 3a

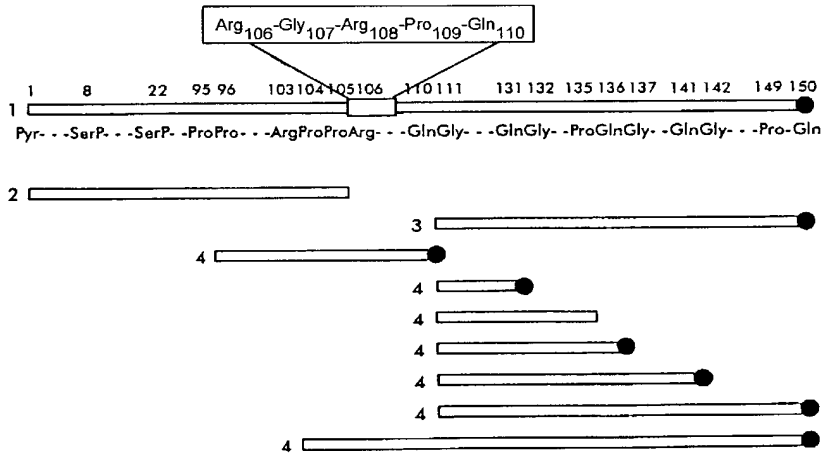
Arg106-Gly107-Arg108-Pro109-Gln110
1 8 22 95 96 103 104 105 106 110 111 131 132 135 136 137 141 142 149 150
1
Pyr- - -SerP- - -SerP- -ProPro- - -ArgProProArg- - -GlnGly- - -GlnGly- -ProGlnGly- -GlnGly- - -Pro-Gln
2
3
4
4
4
4
4
4
4

Fig. 3b

UNITED STATES OF AMERICA COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION	FILE NO. C2432.0044

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that I verily believe that I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural inventors are named) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PREVENTION OF DENTAL CARIES

the specification of which is attached hereto, unless the following box is checked:

☒ was filed on November 16, 2001 as United States patent Application Number or PCT International patent application number 10/009,709 and was amended on November 16, 2001.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information known to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim priority benefits under Title 35, United States Code §119 of any foreign application(s) for patent or inventor's certificate or United States provisional application(s) listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign or Provisional Application(s)

COUNTRY	APPLICATION NUMBER	DATE OF FILING *(day, month, year)*	PRIORITY CLAIMED UNDER 35 U.S.C. § 119
Sweden	9901773-3	May 17, 1999	YES X NO
PCT	PCT/SE00/00930	May 11, 2000	YES X NO
			YES NO

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

UNITED STATES APPLICATION NUMBER	DATE OF FILING *(day, month, year)*	STATUS *(patented, pending, abandoned)*

I hereby appoint customer no. 32172 DICKSTEIN, SHAPIRO, MORIN & OSHINSKY, LLP, and the members of the firm, Edward A. Meilman, Reg. No. 24,735, Gary M. Hoffman, Reg. No. 26,411, Steven I. Weisburd, Reg. No. 27,409, Thomas J. D'Amico, Reg. No. 28,371, Donald A. Gregory, Reg. No. 28,954, Stephen A. Soffen, Reg. No. 31,063, James W. Brady, Jr., Reg. No. 32,115, Jon D. Grossman, Reg. No. 32,699, Mark J. Thronson, Reg. No. 33,082, Michael J. Scheer, Reg. No. 34,425, and Eric Oliver, Reg. No. 35,307, as attorneys with full power of substitution and revocation to prosecute this application, to transact all business in the Patent & Trademark Office connected therewith and to receive all correspondence.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-ou

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